THE SKIN OF PRIMATES

IX. Observations on the Functional Activity of the Sweat Glands in the *Nycticebus coucang* and *Perodicticus potto**

TSUYOSHI AOKI,**† M.D.

Up to the present, a fair number of morphological studies have been made on the sweat glands of primates, but our knowledge of their function is scanty. Recently, Montagna and his colleagues have published a series of papers on the histological and histochemical attributes of the skin of several primitive primates (1–4). Among these, *Nycticebus coucang*, one of the two Oriental Lorisidae, has sweat glands in the hairy skin, all of which are apocrine in type, surrounded by nerve fibers that contain specific cholinesterase (3).

The present paper is largely concerned with pharmacological observations on the sweat glands of *Nycticebus coucang*, together with some observations on one of the African Lorisidae, *Perodicticus potto*.

MATERIALS AND METHODS

Two female *Nycticebus coucang* and 3 female *Perodicticus potto* were used in this study. These animals are not tractable and had to be tranquillized with 1-(1-phenylcyclohexyl)piperidine hydrochloride (Sernyl, Parke Davis), given subcutaneously in doses of 3.5 to 5.5 mg/kg in each experiment. The frontal aspects of the thorax and abdomen, medial or lateral surface of the thigh and lower leg, and volar or dorsal surface of the arm were chosen as test areas. The hair of the area to be tested was clipped with electric clippers. For visualizing the sweat response, the iodine-starch method of Wada-Takagaki was used (5, 6). The following agents were tested for their sudorific effects: L-adrenaline hydrochloride (Parke Davis), L-noradrenaline bitartrate (Winthrop), DL-isopropylnoradrenaline hydrochloride (Isoproterenol, Winthrop), acetylcholine chloride (Merck), acetyl-β-methylcholine chloride (Mecholyl, Merck), carbaminoylcholine chloride (Carcholine, Merck), nicotine (K & K Lab.), and histamine phosphate (U. S. P.). These agents were prepared in 0.9% saline solution at graded concentrations before each experiment, and injected intradermally in volumes of approximately 0.1 ml. Control tests were made with a 0.9% solution of NaCl. Experiments were performed under environmental temperatures of 18° C to 31° C and relative humidity of 38 to 86%.

Skin biopsy specimens, taken from the thigh, lower leg, arm or thorax, were used to demonstrate cholinesterase with the technic of Koelle and Friedenwald (7), as modified by Gomori (8) and Montagna and Ellis (9).

RESULTS

I. *Nycticebus coucang*

Spontaneous sweating. When the animals were not deeply tranquillized, there was some spontaneous sweating on the hairy skin. This was particularly evident and copious on the anticubital region of the arm, where a large group of apocrine glands comprises the *brachial organ* (3). The sweating on this area was readily perceptible even without special methods for visualizing it. Spontaneous sweating, not so conspicuous in other hairy skin areas, seemed to bear no relation to the environmental temperature and was almost absent when the animals were adequately tranquillized.

Sudorific effects of adrenergic and cholinergic agents: Among the agents tested, adrenalin was the most potent, producing definite sweat response in concentrations as low as 10^{-7}. The sweat response usually commenced within one minute after the injection, localized to the injection wheal. If the concentration used was relatively high, sweating gradually spread around the wheal, sometimes showing a diffusion of the response along the lymphatic channels, the extent of spread depending upon the concentration used (Fig. 1). Noradrenalin produced sweat responses almost analogous to those to adrenaline, though the effect was slightly less pronounced. Iso-

* From the Department of Biology, Brown University, Providence 12, Rhode Island.

** Rockefeller Foundation Fellow and Postdoctoral Trainee of the United States Public Health Service at the Department of Biology, Brown University, Providence 12, Rhode Island.

† Present address: Physiological Laboratory, Tohoku University, School of Medicine, Kitayobancho, Sendai, Japan.

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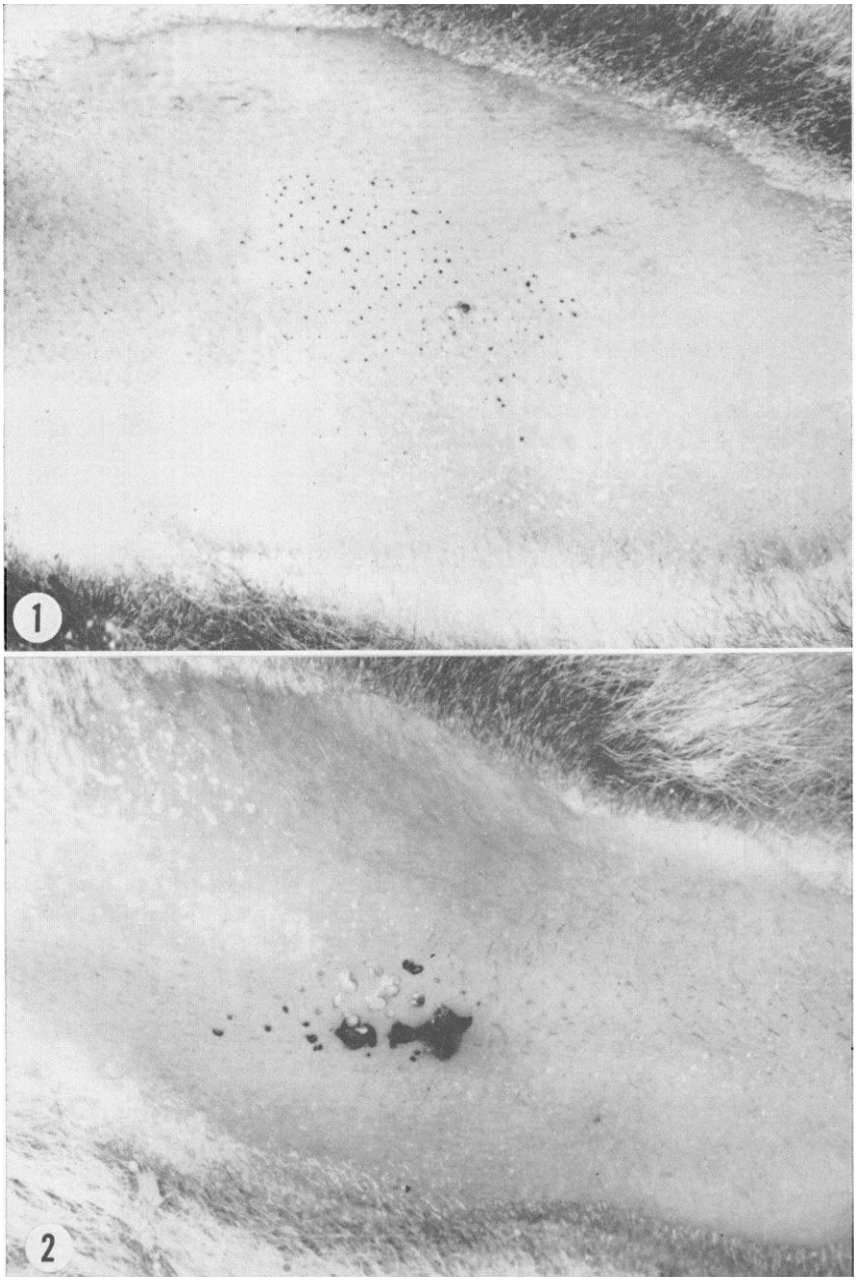

PLATE 1

FIG. 1. Sweat response to intradermal injection of 10^{-5} adrenalin on the medial surface of the thigh of *Nycticebus coucang*. Photographed 10 minutes after injection. (× 1.5)

FIG. 2. Sweat response to intradermal injection of 10^{-6} adrenalin in the anticubital region of *Nycticebus coucang*. Photographed 5 minutes after injection. (× 2)

propylnoradrenalin was practically ineffective in producing sweating in concentrations of 10^{-6} to 10^{-3}.

Acetylcholine, mecholyl, and carcholine were also effective in eliciting local sweat response, but the effects of these cholinergic agents were less remarkable. The sweat spots produced were small and sparse, when compared with those obtained

TABLE I

The threshold concentrations of adrenergic and cholinergic agents for the sudorific effect in the hairy skin of Nycticebus

	Nycticebus I	Nycticebus II
L-adrenaline	$10^{-8} \sim 10^{-7}$	10^{-7}
L-noradrenaline	$10^{-7} \sim 10^{-6}$	10^{-6}
DL-isopropylnor-adrenaline	no effect	no effect
Acetylcholine	$10^{-5} \sim 10^{-4}$	$10^{-5} \sim 10^{-4}$
Acetyl-β-methyl-choline	$10^{-5} \sim 10^{-4}$	10^{-4}
Carbaminoylcholine	10^{-4}	$10^{-4} \sim 10^{-3}$

with adrenalin or noradrenalin. Injections of acetylcholine in 10^{-4} to 10^{-3} often caused a fairly rapid appearance of sweat spots, though sparse, on the outlying area of the injection wheal, while the responses to mecholyl and carbachol in these concentrations were usually confined to the injection wheal.

The minimum effective concentrations of these adrenergic and cholinergic agents for producing a local sweat response were repeatedly examined on the thigh, arm, thorax, and abdomen. The results, summarized in Table I, varied slightly on different days, but no consistent regional differences were found among the areas tested.

Control injections of 0.9% NaCl usually gave no response. Occasionally several sweat spots appeared around the site of the needle prick, especially if the hypodermic needle was not sharp.

Histamine was tested in concentrations of 10^{-5} to 10^{-3}, but it failed to exhibit any sudorific effect.

Intradermal injections of adrenalin or noradrenalin into the skin area of the brachial organ caused very copious sweat responses, adjacent sweat spots often coalescing with one another (Fig. 2). Cholinergic agents were also effective, but the responses were less copious. The sweat droplets were collected easily with a glass capillary tube. The specimens, obtained with either adrenergic or cholinergic stimulations, showed characteristics almost analogous to those in human axillary apocrine sweat (10–12). They were yellowish, viscid, slightly turbid and quick-drying, forming glue-like masses in the glass tube. If the sweat droplets were left intact on the skin surface, they dried and formed a glue-like yellow cap over each follicular opening. The sensitivities of the glands in this area to adrenergic and cholinergic agents were almost identical to those of the glands in other general skin areas.

Effects of dihydroergotamine and atropine on the sweat responses to adrenaline and acetylcholine. In all of the tests, blocking and stimulatory agents were applied to the skin by successive intradermal injections. Five minutes after the injection of dihydroergotamine methanesulfonate (DHE 45, Sandoz) or atropine sulfate (U.S.P.) in volumes of 0.2 ml, adrenaline or acetylcholine in 0.1 ml was administered at the same site. As control, the same concentration of stimulatory agents was injected at the site where 0.9% NaCl had been injected intradermally 5 minutes previously.

DHE in concentrations of 10^{-5} completely annulled the sweat response to adrenalin in 10^{-7} or 10^{-6} and strongly suppressed the response to 10^{-5} adrenalin. Atropine, in 10^{-5} and even in 10^{-4}, failed to exhibit any inhibitory effect on the sweating by adrenalin in these concentrations. The sudorific effect of acetylcholine in threshold concentrations was completely suppressed by 10^{-5} atropine, but not affected by DHE in 10^{-5} or 10^{-4}.

Axon reflex sweating. The intradermal injection of 10^{-5} to 10^{-3} concentrations of nicotine elicited definite local sweating, often showing a fairly rapid and widespread response around the injection wheal, suggestive of the axon reflex nature, though the sweat spots were sparse.

In order to verify whether or not a real axon reflex mechanism is involved in this sweating with nicotine, the single band method of Wada *et al.* (13) was applied. A rubber band about 2 mm in width was tied around the thigh, lower leg, or arm with a tension sufficient to prevent the injected solution from diffusing across the band, and 10^{-5} or 10^{-4} nicotine was injected intradermally near the band. A sweat response appeared not only on the injected side, but also on the opposite side across the band (Fig. 3). The onset of the response on both sides was usually within one minute after the injection, the uninjected side showing a slight delay. The extent and intensity of the response were not always constant, but sweat spots often appeared about 2 cm away from the band, and in maximum responses, over 45 sweat spots were counted across the band. The injection of 10^{-6} nicotine did not cause sweat re-

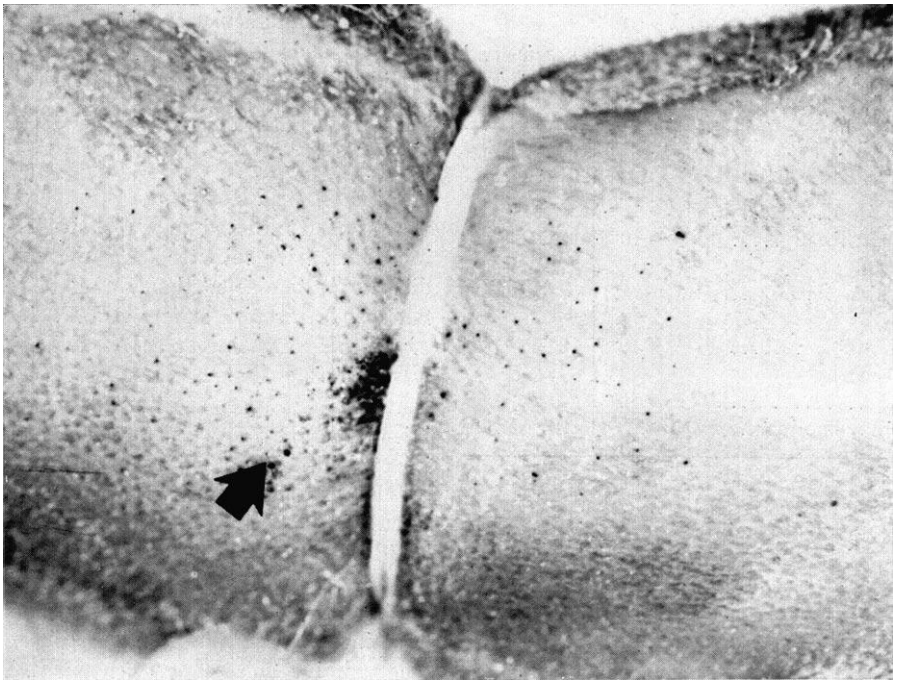

PLATE 2

FIG. 3. Axon reflex sweat response to intradermal injection of 10^{-4} nicotine in the lateral surface of the thigh of *Nycticebus coucang*. Note widespread sweat response around the injection site and across the band. Arrow points to the site of injection. Photographed 10 minutes after injection. (× 2)

sponse across the band, though slight sweating occasionally appeared on the injection wheal.

The effects of procaine hydrochloride (U.S.P.), atropine sulfate (U.S.P.) and hexamethonium bromide (Squibb & Sons) were tested on the axon reflex caused by nicotine, using the band method. In these tests nicotine was mixed together with one of these blocking agents at specified concentrations before each experiment and injected intradermally just distal to the band, the injection of nicotine alone in the same concentration serving as control. A concentration of 10^{-4} procaine definitely suppressed the axon reflex with 10^{-4} nicotine. When the concentration of procaine was increased to 10^{-3}, the inhibition was complete. Atropine as well as hexamethonium, both in concentrations of 10^{-4}, also blocked completely the axon reflex produced by nicotine 10^{-4}.

Acetylcholine was also found to be effective in producing axon reflex sweating. Concentrations of 10^{-4} or 10^{-3} acetylcholine injected intradermally in the thigh or lower leg on either side of the band induced sweating on both sides of the band. The patterns and intensity of the response were comparable to those obtained with nicotine.

Intradermal injections of 4 to 8% NaCl solutions sometimes produced small numbers of sweat spots restricted to the injection wheal, but there was no wide or rapid spread of the response on the outlying area or across the band.

Effect of thermal stimulation. Some of these experiments were made at such environmental temperatures and humidity that the animals often panted. Yet, there was no conspicuous spontaneous sweating on the hairy skin.

The upper half of the body was inserted into a small cabinet heated to about 35° C. There was no sweating produced on the lower half of the body outside of the cabinet, even after one hour, when the animal panted actively.

When a shaved area of skin was exposed directly to radiant heat for 5 or 6 minutes by applying a 60-watt electric light bulb at a distance of about 6 cm above the skin surface, a marked sweating was evoked, always localized to the area heated. Such local sweat response to direct heating of the skin was not affected by procaine or atropine in 10^{-5} to 10^{-3} and DHE in 10^{-5} to 10^{-4}, injected intradermally on the test skin area 5 minutes before the start of heating.

Histochemical demonstration of nerve fibers around the apocrine sweat glands. In agreement

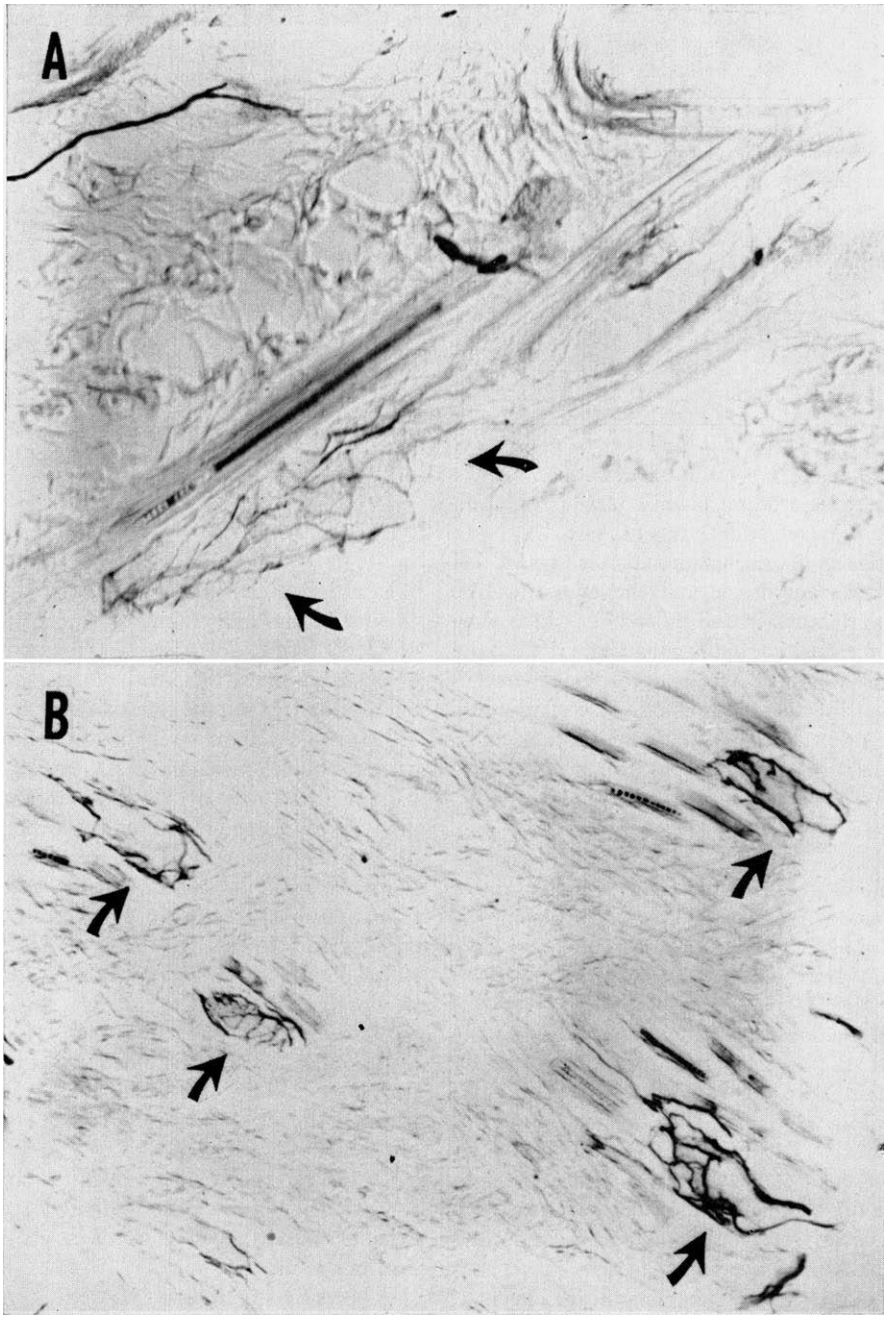

PLATE 3

FIG. 4. Frozen section of the hairy skin of *Nycticebus coucang*, showing cholinesterase-containing nerve fibers surrounding apocrine sweat glands (arrows). A: vertical section of skin from the medial side of the thigh, and B: horizontal section of skin from the medial side of the lower leg.

with Montagna *et al.* (3), the apocrine sweat glands in the hairy skin of *Nycticebus* are surrounded by nerve fibers which contain specific cholinesterase, as illustrated in Fig. 4.

Responsiveness of the eccrine sweat glands in the palms and soles. Animals given small doses of Sernyl always showed some spontaneous sweating on the palms or soles, but those given relatively

TABLE II

The threshold concentrations of the sudorific agents on the palm and sole of Nycticebus

	Nycticebus I	Nycticebus II
L-adrenaline	$10^{-6} \sim 10^{-5}$	$10^{-6} \sim 10^{-5}$
L-noradrenaline	10^{-5}	$10^{-6} \sim 10^{-5}$
DL-isopropylnor-adrenaline	no effect	no effect
Acetylcholine	$10^{-8} \sim 10^{-7}$	$10^{-8} \sim 10^{-7}$
Acetyl-β-methyl-choline	$10^{-8} \sim 10^{-7}$	10^{-7}
Carbaminoylcholine	10^{-8}	10^{-8}

large doses (5 mg/kg or more) had no spontaneous sweating, thus making possible an examination of the sudorific effect of the agents tested.

The glands of the palms and soles responded well to both adrenergic and cholinergic agents. Unlike the glands in the hairy skin, these were much more sensitive to the cholinergic than to the adrenergic stimulations. The threshold concentrations of the agents for eliciting sweat response are given in Table II. Only isopropylnor-adrenaline, tested in concentrations of 10^{-6} to 10^{-3}, gave equivocal or no sweat response.

II. *Perodicticus potto*

The sweat glands of the general hairy skin of *Perodicticus* are also of the apocrine type only, as in *Nycticebus*, but the glands of *Perodicticus* have no cholinesterase-positive nerves around them (1).

Slight spontaneous sweating was occasionally observed on the general hairy skin, especially when the animal was emerging from the anesthesia.

Adrenalin, isopropylnoradrenalin, acetylcholine, and mecholyl were tested on the thigh, chest, and abdomen. All of these agents gave positive local sweat responses except isopropylnoradrenalin, which was ineffective in concentrations of 10^{-6} to 10^{-3}. In general, the effects of these agents were somewhat less pronounced than those observed in *Nycticebus*; they were more variable in individuals and on different days. The apocrine sweat glands of *Perodicticus*, like those of *Nycticebus*, were more sensitive to adrenalin than to acetylcholine or mecholyl. Adrenalin was often effective in concentrations of 10^{-6} to 10^{-5}, whereas the threshold concentrations of acetylcholine and mecholyl were at most 10^{-4} to 10^{-3}.

There was no evidence of the axon reflex sweating. Nicotine was tested in concentrations of 10^{-5} to 10^{-3} on the lower leg or forearm, using the band method described above. There was very slight and inconstant sweating, localized to the injection wheal, but in none of the tests was there a sweat response across the band or in the area surrounding the injection wheal. The response to acetylcholine was also restricted to the site of injection with no rapid spread of sweating characteristic to the axon reflex.

In confirming the report of Montagna *et al.* (1), histological studies showed no nerve fibers around the apocrine sweat glands in the skin of the thigh and lower leg of *Perodicticus*.

DISCUSSION

Intradermal injections of nicotine and acetylcholine in the hairy skin of *Nycticebus* caused rapid, widespread local sweat responses suggestive of the axon reflex, though they were far less pronounced than those produced in the skin of man (13, 14). That these sweat responses are due to an axon reflex mechanism was verified by the band method and by the inhibitory effects of procaine and hexamethonium upon them. On the other hand, there was no evidence of the axon reflex sweating in the skin of *Perodicticus*. These results are consistent with the findings that the sweat glands in the hairy skin of *Nycticebus* are supplied with nerve fibers which contain specific cholinesterase (3) (Fig. 4), whereas those in *Perodicticus* have no such nerves (1). Since the axon reflex sweating is mediated through the peripheral sudomotor nerve fibers (14, 15, 16, 17), perhaps the cholinesterase-positive nerve fibers around the sweat glands of *Nycticebus* are responsible for the occurrence of the axon reflex response. We have hitherto had no convincing evidence of an apocrine sweat response due to axon reflex mechanisms (18, 19, 20). The finding that apocrine sweat glands in a primitive primate are innervated and can be activated by the axon reflex mechanism is interesting from a phylogenetic development of the innervation of sweat glands.

One of the characteristic features of the cholinergic nerves is the presence of the specific cholinesterase in them. The eccrine sweat glands in human skin (21, 22, 23) as well as those in the

footpads of the cat (22, 24) are surrounded by many cholinesterase-containing nerve fibers. However, some sensory nerves, and even adrenergic neurons, are reported to contain some specific cholinesterase (25). If the apocrine sweat glands of *Nycticebus* were cholinergically innervated, they might be expected to be highly sensitive to cholinergic stimulations. On the contrary, they showed the highest sensitivity to adrenergic agents. These results are in essential agreement with those obtained by Montagna *et al.* (3). It must be noticed that high concentrations of NaCl failed to elicit axon reflex sweating in the hairy skin of *Nycticebus*, in spite of the effectiveness of nicotine and acetylcholine. This finding is analogous to the fact that the pilomotor axon reflex in human skin, which is conceived to be mediated through the adrenergic pilomotor nerve (26), could be produced by nicotine and acetylcholine, but not by high concentrations of NaCl (27). Thus, one is tempted to assume that the nerve fibers around the sweat glands of *Nycticebus* are adrenergic.

It is unlikely that the innervation of these glands participate in thermoregulatory sweating, since no sweating was observed on the hairy skin in animals that panted vigorously under high environmental temperature. Yet, spontaneous sweating, though slight and sparse, sometimes occurs on the hairy skin of *Nycticebus*, regardless of environmental temperature. This sweating, particularly clear on the skin area of the brachial organ, which has the richest innervation (3), was abolished by adequate tranquillization, just as was the spontaneous sweating on the palm and sole. Perhaps such spontaneous sweating may be due to an emotional nervous mechanism, probably associated with the cholinesterase-containing nerve fibers around the sweat glands. However, similar slight spontaneous sweating was also occasionally found in the hairy skin of *Perodicticus*, the sweat glands of which are not supplied with cholinesterase-containing nerve fibers. Therefore, one cannot assume that spontaneous sweating in *Nycticebus* is due entirely to a nervous mechanism.

The sweat glands in the palms and soles of *Nycticebus* responded well to both adrenergic and cholinergic agents, the latter being more effective than the former. In this respect these sweat glands are essentially analogous to the eccrine sweat glands in the cat footpads and those in the skin of man. Montagna *et al.* (3) described that the sweat glands in the soles and palms of *Nycticebus* have some morphological and histochemical properties common to both eccrine and apocrine sweat glands. It is of interest here that these glands in *Nycticebus*, like the apocrine sweat glands in the hairy skin, failed to respond to isopropylnoradrenaline which is effective in activating the eccrine sweat glands of man (28, 29).

SUMMARY

The functional activity of the sweat glands in *Nycticebus coucang* and *Perodicticus potto* was studied pharmacologically.

The apocrine sweat glands in the hairy skin of *Nycticebus* responded both to adrenergic agents, such as adrenalin and noradrenalin, and to cholinergic agents, such as acetylcholine, mecholyl, and carcholine, the former being far more effective than the latter. The sudorific effect of adrenalin was selectively inhibited by dihydroergotamine and that of acetylcholine by atropine. The apocrine sweat glands in the hairy skin of *Perodicticus* were also more sensitive to adrenalin than to acetylcholine or mecholyl, but the responses were, in general, less remarkable than those in *Nycticebus*. Isopropylnoradrenalin was ineffective in producing apocrine sweat response in either animals. The sweat glands in the palm and sole of *Nycticebus* were more sensitive to cholinergic than to adrenergic stimulations, but failed to respond to isopropylnoradrenalin.

In agreement with the histological finding that the apocrine sweat glands in *Nycticebus* are innervated by cholinesterase-containing nerve fibers, intradermal injections of nicotine or acetylcholine in the hairy skin were effective in producing apocrine sweating by axon reflex mechanism. The apocrine sweat glands in the hairy skin of *Perodicticus*, which are devoid of such innervation, failed to be activated by axon reflex.

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